Original Article

Oxidative Stress and Homocysteine Metabolism Following Coronary Artery Bypass Grafting by On-pump and Off-pump Techniques

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Received 15 May 2006; Accepted 9 September 2006

Abstract

Background: *It is well documented that coronary artery bypass grafting (CABG) with cardiopulmonary bypass (CPB) causes ischemia and oxidative stress of the whole body. To compare the effect of on – pump and off –pump CABG on the induction of the oxidative stress and the metabolism of homocysteine which is involved in the synthesis of glutathione was investigated in the CABG patients during the early postoperative period.*

Methods: *Plasma homocysteine, folate total antioxidant capacity (TAC) and malonedialdehyde (MDA) were determined by standard methods on blood samples obtained from 40 patients undergoing CABG , preoperatively and at 0,12,48,120 hours and 6 months after surgery ,The patients were divided into two matched groups. One of the groups underwent off - pump and the other on – pump CABG.*

Results: *A marked reduction of homocysteine, folate and significant elevation of MDA were noticed at 0, 12, 48 hours after operation in the both groups (P<0.05). A negative and marked correlation between homocysteine and TAC but a positive and significant correlation between homocysteine and MDA were observed (P<0.05 in the both groups). In CABG operation because of oxidative stress and consumption of GSH (Reduced Glutathione) immediate reduction in the plasma levels of homocyteine occurs in the both techniques. However using off pump CABG induction of oxidative stress and changes in plasma levels of homocysteine are not as high as on- pump CABG.*

Conclusion: *The on-pump technique was correlated with a faster decrease in the homocysteine level during the first 12 hours and with a faster and higher elevation of the homocysteine concentration 12-48 hours postoperatively.*

J Teh Univ Heart Ctr 1 (2007) 39-44

Keywords: *Oxidative stress • Homocyteine • On-pump CABG • Off-pump CABG*

Introduction

Coronary artery bypass grafting (CABG) interventions have been recognized as the main cause of a systemic inflammatory reaction that is believed to result in the global ischemia of the whole body, increased postoperative morbidity, and prolonged hospital stay.[1] Several adverse postoperative outcomes such as renal, pulmonary, and neurological complications, as well as bleeding and multiple organ dysfunctions have been reported.[2-4]

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Delayed recovery to aerobic metabolism contributes to mortality and morbidity[5] and structural and functional derangement,[6] extending the ischemic damage.

Experimental and clinical studies have shown that free radicals are generated in the myocardium during CABG,[7-9] and a great deal of information is now available on the role played by oxygen free radicals in the etiology of myocardial dysfunction after ischemia and reperfusion.[10-12]

Hyperhomocysteinemia is widely regarded as a risk factor for arterial thrombosis,[13] and it is also implicated as a risk factor for venous thrombosis.[14] Homocysteine is a product resulting from the metabolism of an amino acid methionine. Glutathione, which is synthesized from L-cysteine, is considered to play an essential defensive role in the response to oxidative stress. The trans– sulfuration pathway, whose starting substrate is homocysteine, converts methionine to cysteine,[15] which is in turn converted to glutathione. An increased consumption of homocysteine in the human hepatoma cell line hep G2 in response to oxidative stress has been reported.[16] Some investigators have shown that homocysteine levels tend to increase during the first weeks or months after cardiac surgery procedures,[17,18] while they may decrease in general surgical patients early postoperatively.[19]

Recently, off-pump CABG and minimally invasive direct coronary artery bypass have gained widespread use as alternative techniques to conventional on-pump CABG.

The avoidance of CPB and myocardial ischemia – reperfusion has been proposed to significantly reduce the postoperative systemic complications which negatively affect the preoperative course after surgical myocardial revascularization.[20,21]

The present study was designed to investigate and compare the oxidative stress, lipid per oxidation, and homocysteine metabolism following CABG by on- pump and off- pump techniques.

Methods

Forty patients, comprising 33 men and 7 women, with two-vessel coronary artery disease undergoing elective CABG were recruited. We compared 20 patients (16 males and 4 females) who underwent off-pump surgery to 20 patients (16 males and 4 females) who underwent on-pump surgery in a consecutive enrolment design. The mean age of the patients in the off-pump and on- pump groups was 54±9.4 and 56±10.8 years, respectively. Patients with diabetes, diseases of the circumflex and left main stem, valvular diseases, ventricular aneurysm, heart failure, and poor left ventricular function were excluded from the study. Since an altered renal function is known to affect the homocyteine concentration, postoperative acute renal failure (creatinine > 2.0 mg/dl at any time point after the operation) was considered an exclusion criterion.

Ethylene Diamine Tetraacetate (EDTA), blood samples for plasma homocysteine, folate, total antioxidant capacity (TAC), malonedialdehyde (MDA), total protein, and creatinine measurements were obtained from all the patients at hospital admission; at the end of surgery; at 12 , 48, and 120 hours postoperatively; and at 6 months' follow- up. The samples were placed on ice before being centrifuged within an hour, and separated plasma was stored at 70 C° before assay.

The plasma concentration of homocysteine was measured by high-performance liquid chromatography with 7-flouro-2,1,3 benzoxadiazol–4–sulfonate (SBD-F), and fluorescent detection.[22]

The total antioxidant capacity in the plasma samples was assessed using the Randox total antioxidant status kit, and the level of MDA was measured by a spectrophotometrical method,[23] which was partly modified, excluding the high pressure chromatography separation step. The radioimmuno-assay method (Bio-source kit) was used for a folate evaluation; and plasma total protein, creatinine, and glucose were measured with standard methods in the Cobas Mira auto analyzer.

SPSS 11 for Windows was used to perform the statistical analyses. A Paired Student's t-test was employed to determine the significance of the differences between the measured parameters. Comparisons between the two groups were made using ANOVA, and correlations were evaluated by linear regression. Results are expressed as mean± SD, and statistical significance was set at $P<0.05$.

Results

Data from all the patients included in the study were analyzed. The clinical characteristics and operative data are shown in table 1.

There was no hospital mortality, no pulmonary insufficiency, neurologic accidents or incidences of myocardial infarction in the two study groups. Al the patients were extubated between 4 and 14 hours after their arrival in the intensive care unit. The mean time of mechanical ventilator support was 10.8±1.5 hour in the on-pump group, whereas it was only 6.7 ± 0.5 hours in the off-pump group ($P<0.05$). The postoperative blood loss through the chest drains was 642±49 ml in the on pump group and 462±45 ml in the off pump group and the difference achieved statistical significance ($P<0.03$). Blood transfusion was performed when hemoglobin levels fell below , 10g / dl and this was required in 6 cases in on pump group and only in one case in the off pump group. Postoperative fever occurred in 11 patients in the on pump group and only in 4 patients of the off pump group ($P<0.05$). The mean ±SD levels of total antioxidant capacity and MDA after correction for hemodilution, as calculated by changes in plasma protein concentration in the both groups of the patients, are summarized in table 2. The mean of preoperative total antioxidant capacity were similar for both groups. A significant decrement in the mean levels of total antioxidant capacity in the on-pump and off-pump groups was observed at end of operation, 12 h and 48 h after surgery (P<0.05). The serum total antioxidant reached the minimum level 12 h postoperatively and the reduction in on pump group was markedly higher than that of off pump group (P< 0.001). The mean MDA concentrations in the both groups was significantly high at the end of operation 12h and 48 h samples and maximum elevation was noticed at 12 h samples (P<0.05) The elevation in on-pump group was more higher than that of off- pump group (P<0.0001).

The mean±SD values of plasma homocysteine and folate in the both groups at admission in the hospital, soon after end of surgery, 12, 48, 120h and 6 months after the surgery are shown in table 3. A meaningful decrease in both homocysteine and folate was noticed during and several hours after surgery in the off-pump and on-pump groups (P<0.0001 in both cases). The plasma homocysteine levels reached the minimum level 12 hours after CABG and then returned near the baseline levels in the 48 hours' samples. At 12 hours, the reduction in the plasma levels of homocysteine in the on-pump group was significantly higher than that in the off-pump group (P<0.001). The elevation of the homocysteine levels at 48 hours in the on-pump group was markedly faster and higher than that of the off-pump group (P<0.001). No significant differences between the homocysteine levels at 120 hours, 6 months' follow-up, and baseline levels were noticed (P> 0.05 in all the cases). The mean level of folate reached the lowest values 48 hours postoperatively and was still markedly low on postoperative day 5 in both groups. Significant differences were detected between the reduction in the folate levels using the two different techniques of operation (P<0.05).

The correlation between the levels of homocysteine and those of the other measured parameters was determined in the 12 hours' postoperative samples with minimum and maximum changes in the concentrations of the parameters. As shown in Table 4, a positive and significant correlation was observed between homocysteine and MDA. A negative and marked correlation was present between homocysteine and the total antioxidant capacity. No correlation was observed among homocysteine and folate. The correlations between the parameters in the other samples were not significant (P>0.05 in all cases).

Table 1. Patients' clinical characteristics and preoperative data*

Variable	On-pump	Off-pump	P value
Age (Y)	56±10.8	54±9.4	NS
Male / female	17:3	16:4	NS
Hypertension (n)	4	6	NS
No. of grafts / patients	1.5±0.2	1.9±0.2	NS
Ischemic time (min)	37.3±35	38.5±46	NS
CPB time (min)	71.6±6.4	0	<0.05

*Data are presented as mean±standard deviation

CPB: Cardiopulmonary bypass; NS: Non-significant

Table 2. Total antioxidant capacity and MDA levels in both groups of patients*

Time of Sampling	Total antioxidant capacity (mmol /L)		Malone Dialdehyde (mmol/L)	
	Off- pump	On-pump	Off- pump	On-pump
Admission	1.55±0.19	1.49±0.18	2.41±0.40	2.22±0.32
End of operation	1.22±0.15	0.99±0.18	3.60±0.22	6.38±1.35
12 hours	1.15±0.65	0.81±0.05	4.5±0.31	6.8±1.05
48 hours	1.43±0.17	1.26±0.08	4.3±0.63	3.89±0.88
120 hours	1.45±0.15	1.47±19	2.55±0.32	2.25±0.45
6 months	1.58±0.17	1.53±0.13	2.38±0.24	2.15±0.18

* Data are presented as mean±standard deviation

Table 3. Plasma concentrations of homocysteine and folate in the two groups of patients*

Time of Sampling	Homocysteine (μm/L)		Folate (mg/ml)	
	Off- pump	On-pump	Off- pump	On-pump
Admission	16.5±2.4	15.9±1.8	5.1±1.6	4.9±1.7
End of operation	11.8±1.7	8.2±1.2	4.9±1.7	4.8±1.5
12 hours	7.5±0.8	5.1±0.56	3.8±2.1	3.3±1.6
48 hours	12±1.1	14.5±1.5	3.2±1.6	2.8±1.4
120 hours	15±2.0	16.1±2.2	4.5±2.2	4.0±1.6
6 months	16.1±2.8	16.4±1.6	5.5±1.8	5.3±1.2

* Data are presented as mean±standard deviation

Table 4. Correlation analysis between plasma levels of homocysteine and other measured parameters in 12-hour postoperative samples in on-pump and off-pump groups

Parameters	On-pump group		Off-pump group	
	r	P value	r	P value
Homocysteine–Malone Dialdehyde	0.815	<0.05	0.640	<0.05
Homocysteine- Total antioxidant capacity	-0.640	<0.01	-0.621	<0.01
Homocysteine – Folate	0.356	NS	0.220	NS

NS, Non-significant; r, correlation coefficient

Discussion

It has been reported that cardiac surgery- related ischemia-reperfusion injury to the myocardium is caused by the oxidative stress, derived from the formation of reactive oxygen species in the early phases of the reperfusion.[24] CABG with the use of CPB causes ischemia and oxidative stress of the whole body.[25] A more radical and effective way of counteracting the effects of the inflammatory reaction and oxidative stress may be the omission of CPB itself. Elevated plasma levels of the amino acid homocysteine have been identified as an independent risk factor for atherosclerosis.[26] Animal models of hyperhomocysteinemia have shown an altered vascular function, including the promotion of smooth muscle cell growth and the development of atherosclerosis. Several investigators have shown that the homocysteine levels tend to increase during the first weeks or months after cardiac surgery procedures,[18,27] while they may decrease in general surgical patients early postoperatively.[28]

The present study was designed to demonstrate the differences in the oxidative stress and homocysteine metabolism between two groups of patients undergoing on- and off-pump CABG.

In our study, a significant reduction in the levels of the total antioxidant capacity and a marked elevation in the concentration of MDA were noticed in the patients undergoing CABG by on- and off-pump techniques, but the changes in the levels of the parameters in the on-pump group were significantly higher than those of the off-pump group. As stated earlier, CABG with the use of CPB begets the oxidative stress of the whole body, and a markedly greater decrease in the level of the total antioxidant capacity and a significantly higher increase in the concentration of MDA in the on-pump CABG procedure may be partially due to the reperfusion of the heart following global cardiac arrest, oxidative stress, and inflammation.[29]

The oxidative stress following CABG may lead to an increased consumption of GSH and of homocysteine. The oxidative stress increases the activity of gamma–glutamylcysteine synthetase, which catalyses the transformation of L-cysteine to L- glutamate, the first rate limiting step of GSH (Reduced Glutathione) biosynthesis,[15] leading to an increased GSH synthesis in cells. One of the main determinants of the rate of GSH synthesis is the availability of cysteine, which derivates from homocysteine through the trans-sulfuration pathway. Therefore, an ischemia–reperfusion-related oxidative stress may induce a reduction in the plasma levels of both cysteine and homocysteine. In this study, it was noticed that CABG surgery by on-pump and off- pump techniques was associated with a meaningful perioperative reduction in the plasma levels of homocysteine and folate. Because of the high oxidative stress in the on-pump group, the reduction in the levels of the parameters was higher than that of the off-pump group ($P<0.001$). It seems that the high oxidative stress in the on-pump technique instigates a high reduction of homocysteine levels in this group of patients. Increased levels of homocysteine 6 days to 6 weeks after CABG have been previously reported,[17] and it is believed that since homocysteine is a known risk factor for atherosclerosis, these changes may be important to the pathophysiology of vein graft disease. Other investigators have found a similar increase of the homocysteine levels 3-6 months after heart transplantation and speculated that hyperhomocysteinemia following surgery could increase the development of cardiac allograft vasculopathy.[18] A reduction of homocysteine concentrations in the early postoperative period after general surgical procedures has also been reported,[19] and homocysteine is considered a negative acute-phase reactant.

In the present study, a marked postoperative reduction of the plasma homocysteine levels was noticed in patients undergoing the on-pump and off-pump techniques of CABG. The percentage of the reduction

in the on-pump group was meaningfully higher than that of the off-pump group; we found that the homocysteine levels were not different form baseline at 6 months' follow-up in both groups. As regards the on-pump technique, similar results have been reported elsewhere,[30] but our results are not in agreement with those of Jeremy and co-workers at 6 months' follow-up.[27] The discrepancies in the different reports may be due to the differences in the time of sampling and the protocol of the studies.

After the correction for hemodilution, as assessed by changes in the plasma portion, a negative and significant correlation between homocysteine and the total antioxidant capacity and a positive and marked correlation between homocysteine and MDA were noticed in the 12 hours' samples in both groups, suggesting that the reduction in the plasma levels of homocysteine was partially due to the consumption of the amino acid in GSH synthesis, which was faster in the on- pump technique. A marked depletion of endogenous antioxidants has also been observed in the ischemic heart upon reperfusion by others.[31] Our data confirm the observation that a reduced form of GSH acts as a first line of defense against the oxidative stress.

The levels of folate reached the minimum value 48 hours after operation and were still meaningfully low in the 120 hours' samples. Nevertheless, at 6 months' follow-up no difference was observed by comparison with the baseline levels. In our study, unlike the others,[27] the reason why the homocysteine levels reached those of baseline at the end of the 6 months' follow-up may be due to the normal levels of folate in the same samples.

In conclusion, our results indicated that both the on- and off-pump techniques of CABG induced the oxidative stress; however, off-pump CABG did not provoke as much oxidative stress and change in the homocysteine plasma levels as on-pump CABG did. In both techniques, it was the oxidative stress and consumption of GSH that brought about an immediate postoperative reduction in the plasma levels of homocysteine.

The on-pump technique was correlated with a faster decrease in the homocysteine level during the first 12 hours and with a faster and higher elevation of the homocysteine concentration 12-48 hours postoperatively. The data may define one of the beneficial effects of graft operation by the off-pump technique and usefulness of antioxidant supplementary before CABG operation.

Acknowledgments

This study was supported by Tabriz University of Medical Sciences. The authors gratefully appreciate the assistance of the personnel of the Central Laboratory and Research Department of Shahid Madani Heart Hospital, Tabriz, Iran. This study has been approved by the Institutional Review Board of the Tabriz University of Medical Sciences.

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